

Bacteriological and Immunopathological Diagnosis on Morel's Disease

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Preface

This work has been carried out at the department of pathology and microbiology. Faculty of veterinary medicine. University of Khartoum, under the supervision and guidance of professor Sulieman Mohammed El-Sanousi.

Dedication

To my mother, Father and brother with my love.

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Table of Contents

Preface.....	2
Dedication.....	3
Acknowledgment.....	4
List of contents.....	5
List of Figures.....	8
List of Tables.....	9
Abstract.....	10
Introduction and objectives.....	12
CHAPTER ONE : LITERATURE REVIEW.....	14
1.1 Abscess disease of sheep (Morel's Disease).....	14
1.2 Manifestation of the disease.....	15
1.3 Causal agent of Morel's disease.....	18
1.4 Toxins and Enzymes.....	21
1.4.1 Catalase.....	21
1.4.2 Coagulase.....	21
1.4.3 Other Enzymes.....	22
1.4.4 Exotoxins.....	22
1.4.5 Staphylolysins.....	22
1.4.6 Panton-Valentine.....	23
1.4.7 Staphylococcal Enterotoxins.....	23
1.4.8 Exfoliatin or Exfoliative Toxin.....	23
1.4.9 Protein A.....	24
1.5 Taxonomy and Classification of the causal agent of Morel's disease.....	24
1.6. Surface proteins of <i>Staphylococcus aureus</i>	29
1.7 Plaque Forming cell assay.....	31

1.7.1 Direct plaque.....	31
1.7.2 Indirect plaque.....	31
1.8 Pathogenicity Tests.....	32
1.8.1 Pathogenicity of <i>Staphylococcus aureus</i> subsp. <i>anaerobius</i> to laboratory animals.....	32
1.8.2 Pathogenicity of <i>Staphylococcus aureus</i> subsp. <i>anaerobius</i> to sheep and goats.....	32
1.9 Capsules.....	34
1.10 Cellular Components as vaccines.....	35
CHAPTER TWO : MATERIALS AND METHODS	
2.1 Collection of Samples.....	38
2.2 Culture Media.....	38
2.2.1 Liquid media.....	38
2.2.1.1 Nutrient broth.....	38
2.2.1.2 Brain heart infusion.....	39
2.2.2 Solid Media.....	39
2.2.2.1 Blood agar base.....	39
2.2.2.2 Nutrient agar.....	40
2.3 Biochemical Properties.....	40
2.3.1 Catalase test.....	40
2.3.2 Oxidase test.....	40
2.3.3 Coagulase test.....	41
2.3.3.1 Slide Coagulase test.....	41
2.3.3.2 Tube Coagulase test.....	41
2.4 Sugars fermentation.....	41
2.4.1 Indicators.....	41
2.5 Novobiocin sensitivity test.....	42

2.6 Urease test.....	42
2.7 Haemolytic activity.....	42
2.8 Histopathology.....	42
2.8.1 Fixation.....	43
2.8.2 Tissue processing.....	43
2.8.3 Dehydration.....	43
2.8.4 Clearing.....	43
2.8.5 Wax Impregnation and Embedding.....	43
2.8.6 Microtomy.....	43
2.8.7 Manipulation of the section.....	43
2.8.8 Staining of the section.....	45
2.8.9 Mounting.....	45
2.9 The Immunological Test.....	45
2.9.1 The Plaque Forming cell assay.....	45
CHAPTER THREE : RESULTS	
3.1 Clinical Examination.....	48
3.2 Laboratory Examination.....	48
3.3 Pathological Tests.....	48
3.3.1 Pathological test in sheep.....	48
3.3.2 Pathological test in Laboratory Animals.....	49
3.4 Immunological Test.....	49
3.4.1 Plaque Forming cell assay.....	49
CHAPTER FOUR : DISCUSSION.....	
CONCLUSION AND RECOMMENDATIONS.....	
REFERENCES.....	
	65

List of Figures

No.	Figure	page No.
1	Multi abscess in liver	53
2	Abscess in liver	54
3	Suppurative spleenitis	55
4	Lymph node abscess	56
5	Abscess in subcutaneous tissue	57

List of Tables

NO. TABLE

1	Results of biochemical test for <i>Staphylococcus</i> species.....	50
2	Group of rats inoculated by <i>Staphylococcus aureus subsp anaerobius</i>	50
3	Group of rats inoculated by <i>Staphylococcus aureus</i> ...	51
4	Group of rats inoculated by <i>Staphylococcus aureus subsp. anaerobius</i> and <i>Staphylococcus aureus</i>	51
5	Group of rats inoculated by <i>Staphylococcus caseolyticus</i> ...	51
2	Group of rats inoculated by <i>Staphylococcus lugdunensis</i>	51
3	Average of the results of the previous tables.....	52

Abstract

Immunopathological studie on Morel's disease were carried out, using plaque forming cell assay (PFCA).

About forty five samples taken from sheep abscesses for isolation the causative agent. Thirty two isolates were identified as *Staphylococcus* species; sixteen isolates were identified biochemically as *Staphylococcus aureus subsp. anaerobius*, nine isolates as *Staphylococcus aureus*, five isolates as *Staphylococcus epidermidis*, one isolates as *Staphylococcus simians*, and another one as *Staphylococcus cohnii subsp. urealyticus*. The tissue samples collected from sheep with abscess disease for histopathology, showed areas of liquifactive necrosis surrounded by inflammatory cells and fibrous tissue capsule or collection of inflammatory cels predominantly neutrophils and mononuclear cells surrounded by a weak fibrous reaction.

Rats were found to be resistant to experimental infection with *Staphylococcus aureus subsp. anaerobius*, *Staphylococcus aureus*, a combination of *Staphylococcus aureus subsp. anaerobious* and *Staphylococcus aureus*, *Staphylococcus caseolyticus* and *Staphylococcus lugdunensis*. The rats were divided into 5groups each group consist of 3rats.

Group 1 was inoculated by *Staphylococcus aureus subspp. Anaerobius* and the plaque forming cells (PFCs) were found to be 6915.

Group 2were inoculated by *Staphylococcus aureus* and the PFCs were found to be 4583.

Group 3 were inoculated by a combination of *Staphylococcus aureus subspp. anaerobius* and *Staphylococcus aureus* and the PFCs were found to be 14,738.

Group 4 were inoculated by *Staphylococcus caseolyticus* and the PFCs were found to be 7536.

Group 5 were inoculated by *Staphylococcus lugdunensis* and PFCs were found to be 3390.

In this study, the production of plaques was observed in un tanned red blood cells compared with tanned red blood cells. Another important observation is that the synergetic reaction revealed more plaques formed as compared with the summation result of the combination between *Staphylococcus aureus* subsp. *anaerobius* and *Staphylococcus aureus*.

The rats in this study were resistant to experimental infection with *Staphylococcus aureus* subsp. *anaerobius*, *S. aureus*, *S. aureus* subsp. *anaerobius* + *S. aureus*, *S. caseolyticus* and *S. lugdunensis*.

Beside the experiments, samples were collected from naturally infected sheep with a disease for histopathological sections, showed mature abscess of liquifactive necrosis surrounded by inflammatory cells and capsule of fibres tissue, or as a collection of inflammatory cells predominantly neutrophils and mononuclear cells surrounded by a weak fibrosis.

Introduction

Sheep export constitutes a big portion in the exported livestock. Many diseases were known to hinder its export, of these Morel's disease (Sheep Abscess Syndrome) cause a great economical losses especially among sheep fattened for exportation. Many shipments of exported sheep sent back to the Sudan due to disease. The important role of exported lambs in the foreign currency earnings of the Sudan gives control of this disease the urgent priority.

Morel's disease is an endemic non-fatal contagious disease. It is caused by *Staphylococcus aureus subspecies anaerobius* which affected fattened lambs between 8-10 months of age (Morel's,1911). It seems to affected all age groups causing abscesses in animals. The disease has a sudden appearance and it affected most of the flock. The disease characterized by abscess formaton in the superficial lymph nodes, some times the abscess is found in the intramuscular and subcutaneous tissues (Shirlaw and Ashford,1962 and Bajmocy *et. al.*,1984). The size of abscess could be as small as a pigeon egg (Morel,1911 and Shirlaw and Ashford, 1962), or as large as a foot ball (Alhend *et. al.*, 1993). Studies on the aetiology of the disease indicated its relation to increase in the lipids and cholesterol levels in the sera of fattened sheep (Hassan,1996). Any animal showing such abscesses leads to the rejection of the whole exported cargoes thus cause a catastrophic economics loss.

Obejectives :

1. Histopathological studies in sheep naturally infected with Morel's disease.
2. Immunopathological studies using plaque forming cell assay in naturally infected sheep.
3. Immunopathological and histopathological studies in laboratory animals inoculated by different Staphylococcus species inoculated Staphylococcus aureus subsp. anaerobius, Staphylococcus aureus, Staphylococcus caseolyticus, Staphylococcus lugdunensis and a combination of Staphylococcus aureus subsp. anaerobius and Staphylococcus aureus.
4. To compare the production of plaque forming cells between tanned and un tanned red blood cells.

Chapter One

Literature Review

Abscess disease is a contagious suppurative infection of sheep and goats caused by *staphylococcus aureus* subsp. *anaerobius* (Bajmocy *et al.* 1984; Hamad, 1986; El-Sanousi *et al.* 1987; Karamalla 1993; Hassan 1996; Saeed 1995 and Rodwan; 1996) it was first reported by Morel (1911) in France.

The disease is known as abscess disease because abscesses are only signs of the disease (Aynaoud, 1927). The disease also Known as Morel's disease (Shirlaw and Ashford 1962 and Bajmocy *et al.*, 1984). Abscess disease has usually been encountered in 8-10 month old lambs (Morel, 1911).

Subsequently, several other French scientists reported the disease (e.g. Aynaoud, 1922, 1923, 1927; Carre, 1923a, 1927; Bentio and Borrel, 1957). In 1983, the disease was reported in Hungary in a flock of sheep imported from France (Bajmocy *et al.*, 1984). In Spain Loizelier (1958) was the first to report the disease, while Fuente and Suarez (1985) described further outbreaks of the disease in Spain. Afnan and Hedjazi (1978) reported the disease in Iran. The disease diagnosed in Kenya in a flock of sheep which has been built over seven years from endogenous ewes crossed with pedigree corridale rams (Shirlaw and Ashford, 1962) Hamad and El-Sanousi (1989) were the first to describe the disease in sheep in the Sudan. In 1996,

the disease was reported in Denmark in lambs aged 4-5 months of the Lacunae breed 10 days after arrival in Denmark were imported from France.

Goats were considered as naturally resistant to the disease. However, Valenti and Bieler (1984) reported abscess disease in goats from spontaneously developing abscess. The disease was also diagnosed in goats in the Sudan (El-Sanousi *et al*; 1989). In Saudi Arabia, the disease was first reported in goats by Alhendi *et al*, 1993. The disease is commonly known as abscess disease or Morel's disease (Shirlaw and Ashford, 1962; Bajmocy *et al*; 1984).

Manifestations of Disease:

In (1958) Joubert isolated micrococci from lambs affected with abscesses similar to those formerly described by Morel (1911) and Aynaoud (1923). Several workers confirmed the relation of these micrococci to abscess disease (Shirlaw and Ashford, 1962; Bajmocy *et al*; 1984; and Fuente and Suarez. 1985).

Aynaoud (1927, 1928) and Shirlaw and Ashford (1962) found that abscesses due to disease were most often located in the subcutaneous tissues and some times between muscles although. The abscess could be in the lymph node periphery, they were never observed within them. However, Bajmocy *et al*, (1984) and Fuente and Suarez (1985) found abscesses inside the superficial lymph nodes and not around them. Occasionally abscesses were found in the lungs (Aynaoud 1922; Bajmocy *et al*; 1984 Hamad, 1989). K.Moller, J.S.Agerholm, P.Ahrens, N.E. Jensen and T.K. Nielsen (2000) found abscesses were mostly located subcutaneously in the head, neck and shoulder regions close to the regional lymph nodes.

The most common affected lymph nodes were the prescapular, popliteal, inguinal and parotid lymph nodes (Morel, 1911).

Aynaud (1927) and Joubert (1958) considered the angle of the jaw, the shoulder joint and the scrotum to be the predilection sites for the abscesses. Shirlaw and Ashford (1962) mentioned that abscesses occurred in the following order of frequency:

Close to the prescapular, popliteal, parotid and anterior cervical lymph nodes. Bajmocy et al.(1984) noticed that suppuration occurred most frequently in the mandibular, prescapular and subiliac lymph nodes. Fuente and Suarez (1985) observed that the abscesses were most frequently located in the lymph nodes of the mandibular region (mandibular, parotid and lateral retropharyngeal lymph nodes) followed by superficial cervical, subiliac, popliteal, supramammary and scrotal lymph nodes respectively. Hamad (1989) reported that the distribution of suppurative lesions among naturally infected sheep involved the parotid, mandibular, prescapular, popliteal and other lymph nodes respectively.

K.Moller, J. s.Agerholm, P.Ahrens, N.E.Jensen and K.Nielsen, 2000); reported that most lesions were located in association with the superficial cervical lymph nodes followed by parotid lymph nodes and the popliteal lymph nodes. The size of abscess is variable. It could be as small a pigeon's egg or as large as orange (Morel 1911; Shirlaw and Ashford, 1962). Aynaud (1923) mentioned that the size of an abscess was as big as a tow – hand fist. However, Bajmocy et al, (1984) found the abscess to be of the size of a hen's egg or a man's fist, while Alhendi (1993) described the size of a foot ball and (K.Moller, J. s.Agerholm and P.Ahrens, N. E. Jensen and T.K. Nielsen, 2000) demonstrated the abscesses were up to 15 cm in diameter, contained a viscous white – yellow odourless mass, and were enclosed by a 0.2 -0.5 cm thick connective tissue capsule.

The lesions usually start as a small nodule and then gradually increase in size. When ripened, the abscess ruptures and expels thin greenish yellow pus. Healing takes place after a long period of time (Morel, 1911; Aynaoud, 1922; Bajmocy et al., 1985; and Fuente and Suarez.1985) Aynaoud, (1922), however, mentioned that ruptured abscesses might proliferate in other points adjacent to the primary site. More than two abscesses might occur simultaneously in the same animal. Shirlaw and Ashford (1962), and Bajmocy et al., (1984), noticed that adjacent lymph nodes were usually involved a few weeks after those abscesses developing first had ruptured and healed. K. Moller, J. S. Agerholm, P.Ahrens, N. E. Jensen and T. K. Nielsen (2000) demonstrated the abscesses fistulated spontaneously expelling viscous white-yellow odourless pus, most abscesses were closely associated with lymph nodes and the abscess wall was often fused with the lymph nodes capsule by a connective tissue formation that was several millimeters thick. Microscopically, abscesses contained non – structured pus with calcified foci and multiple bacterial colonies surrounded by a homogenous eosinophilic mass. In some lymph nodes, trabecular fibrosis, cortical atrophy and chronic inflammation and fibrosis of the capsule due to compression and involvement by an expanding abscess.

In some cases, the lymph node capsule was located between the lymphoid tissue and the abscess, but in other it was impossible to determinate whether the abscess originated from a lymphadenitis or from a perilymphonodular focus. Lymph nodes draining inflamed areas had an accumulation of neutrophils in the sinuses and depletion of cortical germinal centres. Micro- abscesses or bacterial colonies were not found in any lymph-nodes, but occasionally minor suppurative foci and pulmonary abscesses occurred. Shirlaw and Ashford (1962) reported that histopathological

sections prepared from lymph nodes close to abscesses showed slight hyperaemia of lymphoid tissue, oedema of lymph channels and hyperplasia of lymphoid cords. Hamad (1989) mentioned the hyperplasia of lymphoid tissue and at the centre of each lesion there was a liquefied homogenous material in which poly morphonuclear leucocytes were seen at different stages of destruction with debris of necrotic tissue.

Causal Agent of Morel's Disease

The causal agent was described by Morel (1911) as being micro cocci, arranged in pairs, tetrads and in clusters. Joubert (1958) described the organism as a Gram-positive coccus, 0.6 to 0.8 Mm in diameter, arranged single or groups, non motile, umcapsulated and non-sporing. Shiraw and Ashford (1962) found the coccus to be 1.0 to 1.5 Mm in diameter. Bajmocy *et al*, (1984) showed that cells of the organism were arranged more frequently in tetrads and occasionally in conglomerates. Fuente, Suarez and Schleifler (1985) described the organism of Abscess disease as a Gram – positive coccus 0.8 to 1.0 Mm in diameter, which occurred singly, in pairs and predominantly in irregular clusters and it was non- motile and non-sporing. Hamad (1989) described the organism as Gram-positive cocci, arranged singly, in pairs, in tetrads and predominantly as irregular clusters and non-motile. Hassan (1992) mentioned that the organism as Gram-positive cocci arranged in different manners. The organism was found by El– Sanousi (1989) to be partially acid-fast. Morel (1911), Aynaud (1922) and Carre (1923a,b) mentioned that the organism did not grow on simple media or when incubated aerobically.

Growth and its characteristics:

Shirlaw and Ashford 1962; al Bajmocy *et al*. 1984; Fuente and Suarez (1985) agreed that the good growth occurs when cultures of the

organism are incubated anaerobically or under CO_2 tension, but not aerobically. Carbon dioxide is usually generated by the candle Jar system.

Shirlaw and Ashford (1962) and Bajmocy *et al* (1984) used 10% CO_2 , where as Fuente and Suarez (1985) used (2.5%) CO_2 . Aynaud (1923) reported that organism could grow aerobically when cultivated in egg yolk agar.

Shirlaw and Ashford (1962) showed that the organism did not grow aerobically even after incubation for five days. Bajmocy *et al.* (1984) noticed that the organism was microaerophilic and under aerobic condition pin- point colonies appeared on the fifth day of incubation or later.

Fuente and Suarez (1985) noticed that after a few subcultures on sheep blood agar microaerobically or an aerobically, the organism was adopted to grow aerobically, on sheep blood agar or in Brain Heart Infusion Broth. However, growth was very limited, appearing only as small streaks of confluent growth in those areas that were heavily inoculated. Fuente *et al.* (1985) found that on primary isolation, growth occurred only on media that had been supplemented with blood, serum or egg yolk. They also noticed that no growth occurred when pus sample were directly inoculated into nutrient broth. However slow growth appeared after 48-72hours, as a white granular deposit without turbidity of the medium, when nutrient broth and brain – heart infusion were inoculated from plate cultures.

Shirlaw and Ashford (1962), Bajmocy *et al.* (1984), Fuente and Suarez (1985) and Fuente *et al* (1985) reported that the growth of the organism was slow and appeared after incubation for 48 – 72 hours at 37°C . They described the colonies on sheep blood agar as small, white, circular, smooth, glistening, opaque, low convex and entire. The diameter of colonies was described by Carre (1923 a, b) and Aynaud (1928) to be 1mm, where as

Shirlaw and Ashford (1962) described it to be only 0.5 mm. However, Fuente and Suarez (1985) reported that colonies of the organism arranged between 1 to 3 mm in diameter. There is a general agreement between most authors that *Staph. aureus* subsp. *anaerobius* produces β - haemolysis on sheep blood agar (Shirlaw and Ashford 1962; Bajmocy *et al.* 1984; Fuente and Suarez 1985). This haemolysis has been called ("hot – cold") lysine to emphasize its enhanced haemolytic activity when incubation at 37C is followed by replacement at 4C ambient temperature (Elek and Levy 1950). Hamad (1989) mentioned that the organism produced a wide zone of partial haemolysis around colonies on 5% sheep blood agar. This zone become clear and complete when the culture was further placed at 4 C for 24 hours, while on 5% human blood agar, the organism produced narrow zones of complete haemolysis with sharply demarcated borders. The organism did not produce haemolysis on horse blood agar.

Biochemical Properties:

Generally *Staph. aureus. subsp. anaerobius* is catalase and oxidase negative organism. Fuente and Suarez (1985) reported that the organism produce coagulase, heat labile nucleases, phosphatase, gelatinase and egg yolk factor. However, Shirlaw and Ashford (1962) mentioned that the organism did not produce coagulase or gelatinase enzymes. Bajmocy *et al.* (1984) and Fuente and Suarez (1985) reported that the organism was negative for methyl red and Voges Proskauer (VP) test and did not utilize citrate, produce urease or reduce nitrates to nitrites. However, Aynaud (1928) showed that that organism reduced nitrates to nitrites.

The organism ferment glucose, sucrose, and fructose without gas production but can not ferment mannitol and lactose (Jourbert 1958). Moreover, Shirlaw and Ashford (1962) reported that this organism fermented mannose, but not

maltose but Bajmocy *et al.* (1984) demonstrated that this organism fermented maltose but not mannose.

Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure. Peptidoglycan, a polysaccharide polymer is destroyed by strong acid or exposure to lysozyme. It is important in the pathogenesis of infection, it elicits production of interleukin-1, opsonic antibodies by monocytes and it can be a chemottract. And for polymorphonuclear leukocytes, have endotoxin like activity, produce a localized shwartzman phenomenon, and activate complement.

Teichoic acids, which are polymers of glycerol or ribitol phosphate, are linked to the peptidoglycon and can be antigenic.

Protein A is a cell wall component of many *Staph. aureus* strains that binds to the Fc portion of IgG molecules except IgG3.

Some *Staph. aureus* strains have capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present.

Toxins & Enzymes:

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and their production of many extracellular substance; enzymes, others are considered to be toxins through they may function as enzymes.

A. catalase: Staphylococci produce catalase, which converts hydrogen peroxides into water and oxygen.

B. Coagulase : S.aureus produces coagulase, or clumping factor, on the cell wall surface, that clots oxalated or citrated plasma. The serum factor reacts with coagulase to generate both esterase and clotting activities.

C. Other Enzymes: produced by Staphylococci include a hyaluronidase or spreading factors; staphylokinase resulting in fibrinolysis proteinase; lipases and B- lactomase.

D. Exotoxins: the alphatoxin (hemolysin) is a heterogenous protein that can lyse erythrocytes and damage platelets and has a powerful action on vascular smooth muscle.

Beta toxin degrades sphingomyelin and is toxic for human red blood cells.

Staphylococcus aureus strains may produce as many as 25 – 30 proteins, some of which are associated with pathogenesis. The factors that are either specifically primarily related to *Staph. aureus* are coagulase, staphylolysins, dermonecrotic toxin, lethal factor, Panton-Valentine leukocidin, staphylococcal enterotoxin, toxic shock syndrome toxin-1, and protein A.

Coagulase :

A plasma-clotting protein. A role for coagulase in causing disease has not been discovered, although it is suggested that the coating of the staphylococci with fibrin inhibits their being phagocytosed.

Staphylolysins :

Staphylolysins are hemolysins staphylococcal exotoxins. They also are cytotoxic for cells other than red blood cells such as macrophages, platelets and neutrophils. They can disrupt lysosomes, causing phagocyte to degranulate. They are of several types: alpha, beta, gamma and delta. They differ in the species of red cells that they are able to lyse, in the cations that are required for their activation, in the cell membrane substrates on which they act, in toxic activities and in antigenic composition. The alpha lysin lyses rabbit, sheep and calf red blood cells, the delta lysin lyses human, sheep, rabbit, horse, mouse, rat. And guinea pig red blood cells. Alpha and delta staphylolysins also serve as the dermonecrotic toxin and the lethal

factor of *S. aureus*. The toxins cause necrosis when injected into the skin and death when injected intravenously into laboratory animals.

Panton-Valentine Leukocidin

Many *S. aureus* strains produce a leukocidin different in action and composition from the leukotoxic.

Staphylolyns. Leukocidin increases the permeability of leukocytes to cations and so leads to the swelling and rounding up of the cells. The membranes of cytoplasmic granules fuse with the cytoplasmic membrane of the cell, causing release of the cytoplasmic granules and cell disruption.

Staphylococcal Enterotoxins :

There are six (A.B.C1.C2, D and E) chemically and immunologically related staphylococcal enterotoxins. Under suitable condition of incubation, approximately one Third of *S. aureus* strains shed staphylococcal enterotoxins into food. The toxin can withstand 30 minutes of boiling (it is thermostable) and is not inactivated by the digestive enzymes.

Toxic Shock Syndrome Toxin-1:

This toxin is thought to stimulate the production of interleukin-1 (IL-1) by macrophages. IL-1 has numerous biologic effects, among which are fever induction and the release of acute-phase proteins from the liver. About 90 percent of healthy individuals have protective antibodies against TSST-1.

Exfoliatin or Exfoliation Toxin :

There are two exfoliatins, A and B, one is heat labile and its production is coded for by a plasmid; the other is heat stable and is chromosomally mediated. These exfoliatins are responsible for scalded skin syndrome.

Protein :

Protein A is a surface component of most *S. aureus* strains and it serve as antigen. It is linked to the peptidoglycan layer of the cell wall, but some of it is released extracellularly.

Protein A has the unusual property that the Fc portion of IgG non specifically binds to it, because of this property of binding immunoglobulin is be antiphagocytic by competing with neutrophils for the Fc portion of specific opsonins. Protein A elicits hypersensitivity and inflammation, it injures platelets and it prevents the absorption of bacteriophages (staphylophages) that are specific for *S.aureus*.

Causative agent :**Taxonomy and classification of the causal agent of Morel's disease:**

Morel (1911) described the causative agent as Gram-positive cocci arranged in pairs, tetrads and clusters. Aynaud (1922-1923) observed Gram-positive cocci in films of pus from the affected lymph nodes. Joubert (1958) described *Staph. aureus* as a Gram-positive coccus 0.6-0.8mm in diameter, arranged singly in pairs or in groups, non-motile, non encapsulated and non spring. Shirlaw and Ashford (1962) found it to be 1.0 to 1.5 mm in diameter, Blair et al. (1970) described *Staph.aureus* as spherical, usually occur in irregular clusters, characteristically grape-like but may appear singly, pairs, in short chains or tetrads, 0.5to1.0mm in diameter, Gram-positive, non motile, usually non encapsulated and have no spores. Bajmocy *et al.* (1984) showed that the cells were arranged more frequently in tetrads and occasionally in conglomerates. Fuente et al. (1985) described the organism as Gram-positive coccus, 0.8-1.0mm in diameter, which occurred singly, in pairs and predominantly in irregular clusters and it was non motile and non sporing. Hamad (1989) described the organism as Gram-positive cocci,

arranged singly, in pairs, in tetrads and predominantly as irregular clusters and non motile. El-Sanousi (1989) reported that the organism was partially acid-fast. Karamalla (1993) described the causative agent as Gram-positive coccus, 0.2mm in diameter arranged singly, in pairs and irregular groups. Hassan (1996) described the organism as Gram-positive cocci, arranged singly, in pairs in tetrads and predominantly in irregular clusters. Also he reported that the organism was clumping factor negative, non motile and non-encapsulated.

Morel (1911) Aynaud (1922) and Carre (1923a,b) reported that the organism did not grow on simple media or when incubated aerobically. Carre (1927) separated this coccus from Staphylococci on the basis of its cultural characteristics. But Aynaud (1928) found it shared many biochemical characters with Staphylococci and considered the organism to be *Staphylococcus* adapted to sheep. Bentio and Borrel (1957) and Joubert (1958) considered the causal agent of abscess disease to be an autonomus species that had not yet been described, for which they proposed the name *Micrococcus pyogenes ovis* and *Micrococcus abscedens ovis* respectively. Blanco Loizelier (1958) and Fuente and Suarez (1958) demonstrated that the aetiological agent of abscess disease was a catalase-negative and benzidine - negative Staphylococcus; they considered it to be a respiratory deficient Staphylococcus aureus. However, Fuente et al. (1985) reported that respiratory deficient *Staphylococcus aureus* exhibit a cell wall composition typical of *Staphylococcus aureus* ATCC 126000T. In addition DNA-DNA hybridization indicated that the organism was very closely related to *Staphylococcus aureus* at the species level. However, because of the biochemical distinctiveness and the aetiological importance of the organism, they classified it as *Staphylococcus aureus subsp. anaerobius*. The name

was adopted in the ninth edition of Bergey's Manual of Determinative Bacteriology.

Conventional methods for character determination were first developed and latter used for isolation and characterization.

It was evident from the earlier studies that the novobiocin resistant species *Staphylococcus saprophyticus*, *S. cohnii*, and *S. xylosus* formed a fairly closely related species complex as did the remaining coagulase negative species (Kloos and Schleifer 1975 a,b; Schleifer and Kloos, 1975 and Kloos *et al.*, (1979) validated this complex and showed that Staphylo cocci can be subdivided into several species groups. The *S. epidermidis*, *S. Capitis*, *S. warner*, *S. saccharolyticus*, *S. Caprae*, *S. hominis* and *S. haemolyticus* (Meyer and Schleifer, 1979; Schleifer *et al.*, 1979).

The *S. saprophyticus* group consists of *S. saprophyticus*, *S. cohnii* and *S. xylosus*. The *S. simulans* group consists of *S. simulans* and *S. carnosus*. The *S. scuri* group consists of *S. scuri*, *S. lentus* and *S. hyicus* group consists of *S. hyicus* and *S. chromogens* (Mayer and Schleifer, 1979; Schleifer *et al.*, 1979).

Staphylococcus aureus and *S. aureus* are associated with the *S. epidermidis* group. Where as *S. gallinarum*, *S. Kloosii*, *S. equorum* and *S. arlettae* are related to both *S. epidermidis* and *S. saprophyticus* species group. The *S. delphini* and *S. intermedius* formed a separated group while *S. caseolyticus*, *S. lugdunensis* and *S. schleiferi* can not be accommodated in any of these groups.

Saeed (1995) developed a new scheme for grouping of Staphylococci. Four groups according to biochemical tests were categorized. The coagulase positive group contains *S. aureus*, *S. aureus subsp. anaerobius*, *S. delphini*, *S. hyicus* and *S. intermedius*. In the scheme *S. aureus subsp. anaerobius* was

differentiated from *S.aureus* by not being able to produce pigment, to ferment mannitol anaerobically and V.P negative. It was also distinguished from *S.delphini*, *S. hyicus* and *S.intermedius* by its inability to grow aerobically. The coagulase negative Staphylococci group was divided into two subgroups. The novobiocin resistant group contains: *S.sculri*, *S. equorum*, *S.xylosus*, *S. saprophyticus* and *S. lentus* (Saeed,1995).

The novobiocin sensitive group is divided into two subgroups according to their action on mannose. The mannose negative group contains : *S.warneri*, *S.simulans*, *S.capitis*. *S.capitis subsp.urealyticus*, *S. chromogens*, *S.hyicus*, *S.lugdunensis* and *S.epidermidis*, while *S.caseolyticus* can be accommodated in any of these groups for being coagulase and oxidase negative. The biochemical character can characterize all the species within their groups.(Saeed, 1995). Karamalla (1997). The protein profile of twelve *Staphylococcus* species isolated from sheep abscess *S.aureus subsp. anaerobius*, *S.aureus*, *S.intermedius*, *S.delphini*, *S.hyicus*, *S.saccharolyticus*, *S.carnosus*, *S.chromogens*, *S.simulans*, *S.hominis*, *S.caseolyticus*, *S.haemolyticus*, *S.warneri* visualized by Commassi Brilliant blue stain. Showed four different groups of bands according to their biochemical tests. Group one which contained the coagulase – positive species viz : *S.aureus subsp. anaerobius*, *S.aureus*, *S.intermedius*, *S.delphini*, *S.hyicus* had a large number of protein bands approximately ranging between 21 to 31 bands while the other groups had no more than eight bands.

S: aureus subsp. anaerobius was the only species which had 31 protein bands, and had a unique, very thick protein band of 21 KDa M.wt. Also it had approximately eleven protein bands overlapping with high molecular weight and ranging between 67-43 KDa. These bands were characteristic for *S.aureus subsp. anaerobius* indicating that these cell wall protein may

have a significant role in the pathogenicity of the organism. There was a direct correlation between coagulase production by staphylococci and the number of protein bands.

The protein profiles were not correlated to the cellular fatty acid composition of the staphylococcus species. (Karamalla, 1997).

There was a direct relation between the amount of fatty acids and haemolysin production, which indicates, that there is a direct relationship between fatty acids and Pathogenicity (Karamalla,1997).

There was no relation between coagulase production and cellular fatty acids. These finding suggest that analysis of cellular fatty acids composition could assist in the diagnosis and characterization of many species of staphylococci (Karamalla, 1997).

(Karamalla 1997) reported Morel's disease is caused by several species of the genus staphylococcus. These species have got their unique characters that could identify them from each other. These species included *Staphylococcus aureus subsp.anaerobius*, *S.hyicus*, *S.caseolyticus*, *S.saccharolyticus*, *S.delphini*, *S.scuiri*, *S.hominis*, *S.carnosus*, *S.intermedius*, *S.cohni*, *S.xylosus*.

Surface Protein of *Staphylococcus aureus*:

The structure of the cell wall of Gram – positive and Gram-negative bacteria was determined by electron microscopy using the new technique of freeze substitution method. This enables the analysis of the cell wall structure of staphylococcus aureus in details (Umeda and Amako, 1998). The surface of staphylococcal wall was covered with a fuzzy coat consisting of fine fibers or electron-denes mass (Umeda and Amako, 1998). This coat was completely moved after extraction of eichoic acid from the cell wall with Trichloroacetic acid treatment, but not affected by sodium dodecyl sulfate

or trypsin treatment. It was suggested that considerable amount of teichoic acid was located on the surface of the cell wall and less inside it. The capsule of strain Smith diffuse was assumed to play a role as a barrier protecting from the penetration of antibody against Teichoic acid (Umeda and Amako, 1998).

Pathogenic bacteria often have an arsenal of secreted and surface anchored proteins by which they interact with the host (Patti et al., and Foster et al. 1997). Bacterial pathogens that are primarily extracellular, such as *Staphylococcus aureus* can adhere to components of the extracellular matrix of the host to initiate colonization. Adherence of *staphylococcus aureus* is mediated by protein adhesions of the microbial surface components recognizing adhesive matrix molecules MSCRAMMs (Microbial Surface Components Recognized Adhesive Matrix Molecules) family, which in most cases are covalently anchored to the cell wall peptide glycan (Patti et al., 1994. and Foster et al., 1997). These MSCRAMMs of *Staphylococcus aureus* are fibrinectin-binding protein, clumping factor A (C1fA). Cell wall-anchored surface proteins on Gram – positive bacteria are recognized by analysis of primary amino acid sequences. Five different proteins from *Staphylococcus aureus* have been characterized at the molecular level. Protein A (Spa), the archetypal LPXTG – anchored wall – associated protein, can bind to the Fc domain of IgG and inhibit opsonophagocytosis (Uhlen et al. 1984). Three other proteins, Cna, FnBP and C1fA, are also anchored to the cell wall by the LPXTG motif and clearly promote bacterial attachment to the extracellular matrix (Patti et al., 1994 and Foster et al., 1997). The fifth surface protein is the elastin – binding protein (EbpS) (Park et al., 1996), which is not a member of the LPXTG- anchored family. *Staph. aureus* can bind after host plasma proteins and ECM (Extracellular

Matrix) component, but the bacterial components responsible have not been identified and characterized (Park et al., 1996).

Fibronectin-binding Microbial Surface Components Recognized Adhesive Matrix Molecules (MSCRAMMs).

Fibronectin – binding is a very common property of staphylococcus aureus isolates. Most strains express two related fibronectin – binding proteins, FnBPA, which are encoded by two closely linked genes (Jonsson et al., 1991, Greene et al., 1995). These proteins are responsible for mediating bacterial attachment to immobilized fibronectin in vitro and are important factors contributing to the initiation of foreign body infection (Patti *et al.*, 1994; Vaudaux *et al.*, 1995; Vaudaux *et al.*, 1993).

Hassan (2000). The coated antigen(Ag) on tanned red blood cells(RBCs) showed high percentages of plaque forming cell assay (PFCs) compared to untanned. The PFCA, included antigen of varying nature (Capsule, Toxoid and Whole culture). The protein antigens combined to tanned RBCs while polysaccharide antigens combined to untanned RBCs, indicating that the protein content of the vaccine was more than the polysaccharide contents and hence high induction of the immune response. Leslie and Hay (1980) mentioned that the tanned erythrocyte are suitable coated for most protein antigens. Also the combination of polysaccharide with a protein carrier has been shown to enhance immunogenicity and to stimulate T-cell response. Upon addition anti-sheep IgG more plaques are formed compared to those without anti-sheep IgG. The percentage of PFCs rang from 75-81.59% and 0.42-2% for the vaccinated and non-vaccinated lambs respectively.

The tanned and untanned red cells surrounding the cells secreting specific antibody become coated with the antibody and may be lysed by complement.

Plaque forming cell assay : (Effector – cell Assay):

The basic assay was described by Jerne and Nordin (1963) to detect cells producing antibody against erythrocytes antigen. After the addition of complement the erythrocytes in the locality of the plasma cells are lysed producing holes or plaques in the erythrocytes suspension.

Various methods have been developed for assaying lymphocytes effector functions, including antibody production. Antibody forming cells are measured by plaque forming cells assay, which can detected IgG or IgM producing cells (Ivan Roitt, 1998). Antibody–Forming cells are measured by mixing the test lymphocyte population with antigen–sensitized red cells. Following lymphocytes incubation, the red cells surrounding the cell secreting specific antibody become coated with the antibody and so may be lysed by complement. Two type of plaque can be identified :

Direct plaques:

Antigen–specific IgM antibodies produced by antibody-forming cells are able to directly cause complement–mediated lysis of antigen–sensitized red cells, because of their excellent complement–fixing ability (Ivan Roitt, 1998).

Indirect plaques :

Antigen–specific IgG antibodies do not fix complement so efficiently and so anti-IgG antibodies must be added to enhance the ability of IgG producing cells to lyse the target red cells (Ivan Roitt,1998).

The passive haemolytic plaque assay was used to examine the functional heterogeneity of antibody-producing cells in Salmonid immune organs. An antibody response to vibrio and guillarum antigens was induced by the injection of a somatic antigen extract. This antigen was also coated onto sheep red blood cells (SRBCs) for plaque forming cell(PFC) determination.

The anterior kidney lymphocytes of Coho salmon *Oncorhynchus Kisutch*) possess a much more restricted profile of antibody specificities than do lymphocytes from the posterior Kidney or spleen. This suggests that B cell repertoires differ among the immune organs of salmonids. (Irwin and Kaattari, 1986)

Pathogenicity Tests :

Pathogenicity of *Staphylococcus aureus subsp. anaerobius* to laboratory animals:

Laboratory animals were found to be resistant to experimental infection with *Staphylococcus aureus subsp. anaerobius*. Aynaoud (1928) found that guinea pigs injected intramuscularly or subcutaneously were refractory to experimental infection. Also he found mice to be resistant. Joubert (1958) and Shirlaw and Ashford (1962) found that mice and guinea pigs were refractory to experimental infection irrespective of the route or dose administered. Fuente and Suarez (1985) inoculated four groups of mice intraperitoneally or subcutaneously with increasing doses, and also tried the intramuscular and intradermal routes. They found the mice to be resistant to the disease regardless of the dose or inoculation route. Aynaoud (1928) and Joubert (1958) found rabbits to be resistant to experimental infection through all routes of administration. However, Shirlaw and Ashford (1962) observed cellulitis at the inoculation sites in two out of four rabbits from which the organism was recovered in pure culture. Hamad (1989) found the same findings.

Pathogenicity of *Staphylococcus aureus subsp. anaerobius* to sheep and goats.

Staphylococcus aureus subsp. anaerobius was found to be pathogenic for sheep causing the abscess disease and experimentally for goats (Aynaoud,

1923, 1927 and 1928). Goats seem to have a strong natural resistance to abscess disease, although they are sensitive to experimental infection (Aynaoud, 1923, 1927; El Sanousi *et al.*, 1989). Aynaoud (1927) found that the organism was pathogenic for sheep and goats when administered by the intramuscular, subcutaneous, intraperitoneal and intratesticular routes. Abscesses developed at the inoculation sites, while following intraperitoneal injection of the organism led to development of the abscesses in the abdominal muscles. The organism was not pathogenic when given by the oral or the intratracheal routes.

Shirlaw and Ashford (1962) reproduced the disease in sheep when the organism was administered by the intradermal or subcutaneous routes. Abscesses developed after two days on animals infected intradermally and after 12 days on those infected subcutaneously. Abscesses were observed in adjacent lymph nodes in necropsied animals six weeks following infection. Shirlaw and Ashford (1962) noticed that scarification and rubbing of the organism did not reproduce the disease in sheep. However, Bajmocy *et al.* (1984) were able to reproduce the disease by rubbing the organism in the scarified area, as well as by the intramuscular and intravenous inoculation. Hamad (1989) observed that sheep inoculated with a culture suspension of the organism developed a local abscess, which ruptured on the ninth day of the inoculation, together with a pulmonary abscess in the lung of the same side of inoculation. On necropsy seven weeks following infection, thickening and cording of lymphatic vessels from the skin lesion to the adjacent prescapular lymph node, were the main lesions noticed. Hassan (1996) found scarification to cause multiple micro abscesses in liver, mediastinal lymph nodes and no evidence of abscess formation was observed in the superficial lymph nodes. On the other hand, the internal

organs of fattened sheep inoculated intravenously with viable *Staphylococcus aureus subsp. anaerobius*, showed no apparent lesions. Subcutaneous injection of *Staphylococcus aureus subsp. anaerobius* in the neck region near the parotid lymph node and subcutaneously in the inoculation sites, together with inflammation of the right prescapular lymph node (side of inoculation). Most of the abscesses were become opened on burst by day eight (Hassan, 1996).

In study by Santa-Quiteria, et al., (1994) abscess disease was reliably reproduced in all 5 kids and 4 lambs experimentally infected by rubbing superficial skin incisions with a swab soaked in an overnight culture of *S. aureus subsp. anaerobius*. The clinicopathological changes of this experimental infection were similar to those described in the natural disease suggesting that the primary portals of entry for the bacteria are abrasions or minor wounds of the skin

Capsules :

Karakawak et al. (1974, 1979) demonstrated that the clinical isolates of *S.aureus* grown on appropriate media were encapsulated. These encapsulated strains were resistant to phagocytosis by polymorphonuclear leukocytes and produced extracellular polysaccharides different from teichoic acid.

Some *Staphylococcus aureus* strains are encapsulated and are more virulent to animals in vitro (Mamo et al., 1991). Jawtez *et al.*, (1995) reported that these capsules inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies were present.

These capsules exist either as a macro capsule or a micro capsule. The macro capsule is a stable polysaccharide layer closely associated with the cell wall and not removed by continuous washing or subculture, and is

formed independently of the medium used and is revealed by negative staining (Wilkinson, 1983).

The micro capsule is a polysaccharide layer which is detected by transmission electron microscope (Watson and Prideaux, 1979; Watson and Watson, 1989; and Watson, 1991).

The availability of monoclonal and polyclonal antibodies to capsular polysaccharide allowed the identification of eleven serovars (KaraKawa, et al., 1985, and Sompolinsky, et al., 1985). The microcapsule consist of repeated sequence of hexosamine and uronic acid (Fourniern, et al., 1989).

Cellular Component as Vaccines :

Surface polysaccharide may serve both as virulence factors and as protective antigens for bacteria whose invasion of blood is their primary event (Robbins *et al.*, 1980). These surface antigens are capsular polysaccharides of Gram – positive bacteria. When purified most of these polysaccharides elicit antibodies in healthy adults. The capsular polysaccharide has immunological properties. Other potentially protective immunogenic procedures against *S.aureus* infections include (CPS) as vaccines. The major virulence factor of *S.aureus* is the development of expolysaccharides capsules in vivo, which inhibits the recognition of antibodies to highly antigenic cell wall by neutrophils, to circumvent this inhibition, an attempt was made to produce anticapsular antibodies. Surface polysaccharide capsules formed by pathogenic bacteria are important virulence factor, immunity directed towards capsular antigens is often protective (Foster, 1991). Combination of polysaccharide with a protein carrier has been shown to enhance immunogenicity and stimulate T.cell response (Robbins and Schneerson, 1990). There is also strong evidence indicating anticapsular polysaccharide (CPS) antibodies promote

phagocytosis and Killing of bacteria with microcapsules (Faltom *et al.*, 1990). However, these studies are still at a rather early stage and are particularly limited by the fastidious and largely variable requirements for optimum capsule development by different strains or ecotypes of *S.aureus subsp. anaerobius*. Immunization with protein A might be expected to reverse the inhibition of opsonophagocytosis.

Nickerson *et al.*, (1991) evaluated protein A vaccine and commercially available bacterin (Stomatostaph.) in 10 cows which were challenged later with *S.aureus*. After three lactation trials. There was also reduction in the number of new infections with *S.aureus* in vaccinated cows. However, the number of resolved infections was significantly higher in cows vaccinated with protein A vaccine or bacterin compared with non-vaccinated. A study on vaccination of sheep against Morel's disease has been carried by Rodwan *et al.*, (1996). The vaccine used contained the capsule, toxoid and formalized whole culture given as 1 ml subcutaneously and boosted after two weeks.

The challenge was carried out after one month from the first vaccination. The mentioned vaccine gave protective responses detected by prevention of abscess formation in challenged lambs. The challenged dose was 1200 bacteria (3 times the abscess causing dose reported by Hassan (1996)). The passive immunity transfer from ewes to their lambs was monitored from birth to seventh months of age in those borned from vaccinated ewes against Morel's disease and other borned from non-vaccinated ewes. In the lambs the titres of IgG1 in the first days of the life are in all cases especially high (Verdouw – Chamalaun *et al.*, 1997). This is on vaccinated lambs fed colostrum from their vaccinated ewes acquired a protection of up to nearly 5 months as judged by the high phagocytosis effect, after that the maternal

immunity weaned out. The maternally derived antibodies protected the newborn during the first months of life against some common infections. This type of protection usually lasts four to six months of age after which the offspring develops his own antibodies due to developing of its lymphoid system (Shouman, 1982). The vaccination of lambs after five months gave immunity for the lambs and hence differentiated between the vaccinated and non-vaccinated lambs when challenged at seven months of age. It is obvious that the anti-*Staphylococcus aureus sub sp. anaerobius* IgG was fed in the colostrum after birth up to weaning. Vaccination of ewes increased lambs serum antibody concentration nearly up to the fifth months and then declined compared with lambs reared by unvaccinated ewes. It is suggested that vaccination of ewes before lambing provided passive protection to lambs during the first 20 weeks of age. Thereafter, antibodies in the vaccinated lambs born to vaccinated ewes decreased at the fifth month. The minimum abscess-causing dose for ewes was determined in a challenged ewes, it was found in ewes increasing about ten times that of rams (400:480.000). (Hassan, 2001).

This vaccines prevent staphylococcal infections by interfering with bacterial virulence, but not with bacterial growth. This tactic of neutralizing the activation virulence might serve to maintain in a less virulent state, in such away that, the innate immune defenses of the host are better suited to cleaner it.

Materials and Methods

The pus samples were collected from the affected sheep at Alkadaro and Al Sabaloga slaughters, from the affected lymph node were kept in ice on thermos pot transported to the laboratory for bacteriological examination.

Materials used were sterile scalpels, forceps and universal bottles. Small incision was made and pus was gently squeezed.

Pus was collected aseptically in the sterile bottle which was then submitted for bacteriological examination, direct smears were made from each pus sample fixed by heating, stained with Gram's method and examined microscopically for presence of the causative agent. Pus was streaked on 10% sheep blood agar plates, the remainder of each sample was stored at -20°C for future use. The plates were incubated in an anaerobic jar under increased carbon dioxide tension (candle jar system) at 37°C for 48 hours. The causative agent of Morel's disease was identified and characterized according to the procedures described by Cowan and Steel, (1985).

Culture Media :

Liquid Media :

Nutrient Broth (Oxoid)(g/l)

Lab. lemco powder	1g
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Yeast extract	2g
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Peptone	5g
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Sodium chloride	5g
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(PH 7.4)

Thirteen grams of nutrient broth were dissolved in 1000 distilled water and the PH was adjusted to 7.4 The medium was then distributed i5ml volumes in test tubes and sterilized by autoclaving at 121°C for 15 minutes.

Brain Heart Infusion: (Oxoid)	(g/L)
Calf brain infusion solids	12.5g
Beef heart infusion solids	5.0g
Proteose peptone	10.0g
Glucose	2.0g
Sodium chloride	5.0g
Di-sodium phosphate	2.5g

(PH 7.4 +0.2)

Thirty seven grams were suspended in 1000ml of distilled water, mixed well and distributed in test tubes and sterilized by autoclaving at 121°C for 15 minutes.

Cooked Meat Media (CMM):

The medium consisted of (%w/v) minced meat (500gm) the fat was skimmed off boiled in 500cc distilled water for 20 minutes and strained through muslin by pressing, then spread on a sheet of filter paper to dry. 0.5gm peptone was followed by 0.25gm sodium chloride and the soup filtered from the minced meat. The mixture was then steamed for 20 minutes and then 1ml of HCL was added and the solution was filtered. The PH was adjusted to 7.7-7.8. The nutrient broth was added to bijou bottles each containing about 2.5gms of the dry minced meat. The medium was then autoclaved at 121°C for 20 minutes.

Solid Media:

Blood Agar Base No. 2: (Oxoid) (g/l)

Proteose peptone	15g
Liver digest	2.5g
Yeast extract	5g
Sodium chloride Agar No. 3	12g

PH 7.4 (approx)

Forty grams were suspended in one liter of distilled water, brought to the boil to dissolve completely, mixed and sterilized by autoclaving at 121°C for 15 minutes. Then cooled to 45-50°C before addition of 7% defibrinated ovine blood, mixed gently and dispensed onto sterile petri-dishes in 15ml portions each.

Nutrient Agar:

Nutrient agar was prepared by adding twenty eight grams (Oxoid CMR₃₂) were dissolved in 1000 ml of distilled water and boiled to dissolve completely, mixed and sterilized by autoclaving at 121°C for 15 minutes. After cooling to 45°C, defibrinated sheep blood was aseptically added to a final concentration of 10%. The medium was then poured onto sterile plates in 15ml aliquots and left to solidify under complete aseptic condition.

Biochemical Properties:

All biochemical tests were performed according to the procedures described by Cruick Shank et. al. (1975) and Cowan and Steel (1993). Tests were performed with 48hrs old cultures on 10% sheep blood agar plates and incubated under micro aerophilic condition at 37°C.

Catalase test:

A drop of 30% aqueous solution of hydrogen peroxide (H₂O₂) was placed on a clean microscope slide. A small amount of the culture was picked with a clean thin glass rod and placed on the hydrogen peroxide. The production of gas bubbles from the surface of the culture material was considered as a positive reaction.

Oxidase Test:

Strips of filter papers What man's No. 1 soaked in 1% solution of tetra methyl-p-phenylene diamine dihydrochloride. After draining for about 30 seconds, the papers were freeze dried and stored in a dark sterile screw-

copped bottles. For use, a strip was laid with a sterile forceps on a clean petri-dish and moistened with sterile distilled water. Colonies from a fresh young culture of the test organism (18-24hrs old) were picked with a sterile bent glass-rod and rubbed on the filter paper. Within 5-10 seconds, a dark purple colour developed and this was considered as positive reaction.

Coagulase Test:

Slide coagulase test:

A clean microscopic slide was used to carry the test by emulsifying about two colonies of test culture in a drop of normal saline. Then a loop full of undiluted rabbit plasma was added to the drop of bacterial suspension. Within 5-10 seconds coarse visible clump was recorded as a positive result.

Tube Coagulase Test:

One ml of fresh sterile rabbit plasma was diluted 1:10 with sterile normal saline was placed in each of a series of small sterile agglutination test tubes, that were inoculated with the test organisms. A drop of broth was added to each of the tubes which were then incubated at 37°C. The tubes were examined for coagulation after 1, 2 and 6hrs. Tubes in which coagulation occurred were considered positive. Negative tubes were left after further incubation over-night occurred and re-examined them where they will be un-coagulated. Control positive, negative and un-inoculated were set with the tested cultures.

Sugars Fermentation Test:

The test culture was inoculated in the peptone water sugar and then incubated tubes were examined after 48hrs frequently for up to 14days. Change of colour to pink indicate positive result, where as gas production was indicated by development of an empty space in the Durham's tube.

Noviocrin:**Novobiocin sensitivity test:**

Novobiocin disks (BBL and sensi-disc) were used. After streaking the organism lightly on blood agar. The disks were added gently on the surface, then indicator.

Andrate :

The indicator is composed of 5gm acid fuchsin, 100ml DW and 150ml. N-NaOH. The acid fuchsin was dissolved first in DW, then 150ml of alkaline solution was added, mixed and allowed to stand at room temperature with frequent shaking for 24hrs. The colour should change from red to brown (Cowan and steel, 1993).

Incubated for 24hrs under increased CO₂ tension, then the area of inhibition was recorded as sensitive or resistant.

Urease Test: (Christensen's 1946):

Urea agar slope was heavily inoculated with test culture and incubated for 48hrs. urease hydrolysis urea into ammonia and carbon dioxide A positive reaction was indicated by the change of the 14days before they were considered final.

Haemolytic Activity:

Haemolytic activity of the different species of the genus staphylococcus was preliminary checked by the examination of the blood agar plates after incubation.

The clear zone of haemolysis was noticed and the plates were then kept at 4°C (Hot-Cold haemolysis) for 24hrs (B. haemolysis).

Histopathology:

Preparation of the tissue for histopathological examination was carried out according to the method described by Drury and Wallington (1980).

1-Fixation:

About one cm³ of sample was fixed in 10% formal saline so as to preserve and fortify the tissues. The tissues were fixed in the formal saline for at least three-day.

2- Tissue processing:

Tissue processing was performed automatically using Elliot tissue processor. The formalin fixed tissues were labeled and immersed in 70% alcohol for 15 minutes before it was introduced to the processing machine.

3- Dehydration:

Paraffin would not be penetrating the tissue in the presence of water. So dehydration was essential in the preliminary process. That was done using different ascending grades of ethyl alcohol varying between 70% to absolute alcohol to prevent the distortion that would accompany the direct transfer of tissue from 10% formalin to absolute alcohol.

4- Clearing:

Decline and chloroform were used as clearing agents.

5- Wax impregnation and Embedding:

Tissues were embedded in paraffin wax of a melting point 54°C to 56°C in moulds of suitable size then the blocks were allowed to cool and solidify.

6- Microtomy:

Microtomy of the embedded tissues was performed using a rotatory microtome (Baired and Tatlock Ltd., England). The sectioned produced were 4-6 microns in thickness.

7- Manipulation of the section:

The sections were fixed on a glass slide and dried at 37°C the hours.

8- Staining of the section:

Before staining the section was freed of the wax using xylene and rehydrated using different descending grades of alcohol ranging from absolute to 70% alcohol.

Then the tissue were cleared with xylene and stained with eosin and haematoxylin.

9- Mounting:

The staining sections were covered with glass cover slips which were fixed by Canada balsam. The sections were all allowed drying for 24 hours before they were examined.

The immunological methods.

The plaque forming cell assay:

This assay was used to quantitates the number of antibody producing cells in an organ. The basic assay which we will describe was developed by Jerne and Nordin to detect cells producing antibody against erythrocyte antigens. Spleen cells from immune mice are incubated with the immunizing erythrocytes. After the addition of complement the erythrocytes in the locality of the plasma cells are lysed producing holes or plaques in the erythrocyte suspension.

Preparation of Antigen (Ag):

Tanned and un tanned Red blood cells preparation:

Sheep blood was collected in Alsever's solution (10%). Then sheep red blood cells (SRBCs) were washed 3 times with phosphate buffer solution (PBS), pH 7.4.

Experimental animals:**Experimental design:**

Fifteen rats were grouped into five groups and inoculated with different species of *Staphylococcus*. The inoculated doses were 4.5×10^7 CFU/ml for *S.aureus subsp. anaerobius*, 2.7×10^7 CFU/ml for *S. aureus*, 2.113×10^7 CFU/ml for a combination of *S. aureus subsp. anaerobius* and *S.aureus*, 7.7×10^7 CFU/ml for *S.caseolyticus* and 3.6×10^7 for *S.lugdunensis* by intraperitoneal (I/P) and subcutaneously (S/C) routes.

The viable count:

Total viable count method (Miles and Mizra, 1938) was used to detect the number of viable organisms in one millilitre of culture. The organism were cultured in brain heart infusion and then serially diluted in 0.1% peptone water broth.

Experimental design:

Equivalent volumes of 10% SRBCS and tannic acid solution (5mg tannic acid in 100ml PBS) were mixed and incubated at 37°C for 20 minutes. At the same time un tanned SRBCs were washed once. The Ag (vaccine) total protein contents was determined by Biurttte method (king and wotton, 1969). The Ag (vaccine) was dissolved in PBS PH 6.4 at concentration of 0.2 mg/ml. Equivalent volumes of AG, with either tanned or un tanned sheep RBCs were mixed well and incubated at 37°C for 20 minutes. The combination was then washed three times in normal saline containing 0.57 heat inactivated normal rabbit serum (at 56°C).

(i) Sheep Red Blood Cells (SRBCs):

These were taken from sheep blood and washed in Hank's saline (Balance salt solution "BSS"), three times at 300g for 1 min. Adjust to 20% v/v.

(ii) Effectors Cells:

Five groups of rats were tested each group contain three rats and the spleen was taken to run the test. The fresh spleen of each rat was taken separately and weighted. The cells were teased through wine mesh and the cell suspensions were collected and the red blood cells were lysed with 0.8% NH_4Cl . Then each suspension was washed 2 times in HBSS (Hank's solution "Balanced salt solution = BSS") at 15000RPM for 10 min at 4°C. The cells resuspended in 5ml of PBS. Mixed 0.1ml of each cell suspension with 0.1ml of 0.2% tiypan blue solution and incubate at room temperature for 5min. Cells were counted using haemocytometer chamber. The number of cells was adjusted to 10^{-7} cell/ml (Leslie and frank, 1980).

The calculation was done as follows:

It is convenient to use x40 objective and to count central, triple-ruled area of the haemocytomater (this area used for red cell counting in haematology.

Number of viable lymphocytes ml^{-1} =

Number of lymphocytes counted $\times 25 \times 10^4 \times$ original dilution (if any)

Number of triple-ruled squares:

(iii) Agarose : The agarose was dissolved in BSS at 0.5% in a 100°C water bath and then hold at 47°C until needed.

(iv) complement: Guinea pig serum was used for the test and diluted 1/5000 in BSS and kept cold all over the test.

(VI) Balanced salt solution (BSS).

Phenol 010mg

CaCl_2 140mg

NaCL 008gm

KCL 100mg

MgSO₄-7H₂O 200mg

MgCL₂-6H₂O 200mg

These ingredients were dissolved and made up to one liter, after adjusting pH to 7.0-7.2 and then the solution was stored at -20°C.

(vii) **Small tube tubes** were placed in 47°C- water bath and to each tube the following were added:

Agarose	300ML	600ML
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ABCs	020ML	040ML
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Spleen cell suspension	100ML	200ML
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Complement)1/3dil	040ML	
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This was added while whirl mixing and then all these were mixed rapidly on a whirl-mixer and poured the suspension onto a slide. The slide were incubated in a humidity chamber at room temperature overnight. Then they were read by both macroscopic and microscopic (low power lens x), every half an hour up to three hours and then left overnight.

Result

3-1. Clinical Examination:

Forty-five samples of abscesses were collected from EL Kadar and ELsabaloga abattoirs, the common sites of abscesses were parotid, prescapular, sub mandibular lymph nodes and frequently in sub cutaneous tissues.

3-2. Laboratory Examination:

3-2-1. Microscopic and cultural examination:

The collected pus sample cultured on 10% sheep blood agar under increased CO₂ tension, incubated for 48hrs at 37°C and the samples were smeared, fixed and stained with gram's stain and examined under microscope showed thirty isolates that were gram positive cocci arranged singly, in pairs or in groups. The colonial morphology of the species showed small, white colonies with partial or complete haemolysis, thirteen samples contained mixed colonies of gram-positive cocci and pleomorphic bacilli.

Sixteen isolates were identified biochemically as *Staph aureus spp. anaerobius*, five isolates were identified as *Staph. epidermidis*, nine isolates were identified as *staph. aureus*, one isolate was identified as *S. simians* and one isolate was identified as *Staph. C ohnii sub spp. urealyticus* according to their biochemical reactions (Cowan and Steel).

Pathological Tests:

Sheep :

The histopathological changes that were observed in *S. aureus sub spp. anaerobius*, *S. aureus* and *S. Simians*.

Different organs from different isolates showed suppurative inflammation and abscess formation of liquefactive necrosis with a pinkish color surrounding by inflammatory cells; macrophages and neutrophils and

capsule, or abscesses from of collection of neutrophils with very weak capsules. Spleen suppurative inflammation, or with out capsulation and in suppurative splenic.

The abscess showed within the spleen tissue as a zone of neutrophils surrounded white pulp and separated white pulp from red pulp.

The gross appearance showed a yellow colour and homogenous of area of abscess pus surrounded with a capsule of connective tissue some of them were classified. The abscesses found in liver, spleen, lung, lymph nodes, subcutaneous tissue of the neck and abdominal regions.

Pathological test in experimental animals:

Novobvious gross lesion were seen in different organs of the infected rats with different species of *Staphylococcus*, *S. aureus*, *S. aureus sub spp. anaerobius*, *S. caseolytius* and *S. lugdunensis*.

Immunological Test:

Plaque forming Cell Assay (PFCA):

Groups of rats were infected by different species of whole culture of *Staphylococcus aureus sub spp. anaerobiues*, *S. aureus*, *S aureus sub spp. anaerobius* and *S. aurous*, *S.caseolyticus* and *S.lugdunensis*, the plaque formed were counted and showed in table 1, table 2, table 3, table 4 and table 5 respectively. The average of these tables were showed in table 6. In another experiment cross infection was conducted using *S. aureus sub spp. anaerobius* and *S. aureus*, the synergistic reaction revealed highest number of PFCs compared to the results of infection of each bacteria separately as showed in table 6. A high number of PFCs obtained in the group infected by *S.caseolyticus*, where as the lowest number obtained in *S.lugelunensis* infected group as showed in table 6.

- The antigen (Ag) on un tanned red blood cells showed positive result of PFCs compared to coat Ag on tanned red blood cells.
- No results were obtained when using live *Staphylococcus aureus* as antigen compared with *S. aureus sub spp. anaerobius*.

Result of plaque forming Assay (PFCs) in experiment Animals each group consist of 3 rats.

Exp. (1):

Group of rats inoculated by 4.5×10^7 of *Staphylococcus aureus sub sp. anaerobius*.

Table (1):

Rat weight	Spleen w.	Viable lymphocyte	Plaque. F.C in 3 ml	Dead lymph
245	0.599	68,700	8340	1800
244	0.571	46,800	5410	2850
236	0.492	30,150	6700	3750

Exp. (2)

Group of rats infoculated by 2.7×10^7 of *Staphylococcus aureus*.

Table (2)

Rat w.	Spleen w	Viable lymph	PFC/3ml	Dead lymph
243	0.567	9600	5370	3300
233	0.445	9000	3900	750
231	0.417	7200	4485	1200

Exp. (3)

Group of rats inoculated by 2.13×10^7 of *Staphylococcus aureus subsp. anaerobius* and *Staphylococcus aureus*

Table (3)

Rat w.	Spleen w	Viable lymph	PFC/3ml	Dead lymph
249	0.638	21,450	11,820	1950
248	0.624	1650	17,625	1650
239	0.533	22,200	14,768	2700

Exp. (4)

Group of rats inoculated by 7.7×10^7 of *Staphylococcus caseolyticus*.

Table (4)

Rat w.	Spleen w	Viable lymph	PFC/3ml	Dead lymph
256	0.712	50,000	8250	1650
244	0.582	8,700	8145	1050
233	0.446	12,750	6218	1350

Exp. (5)

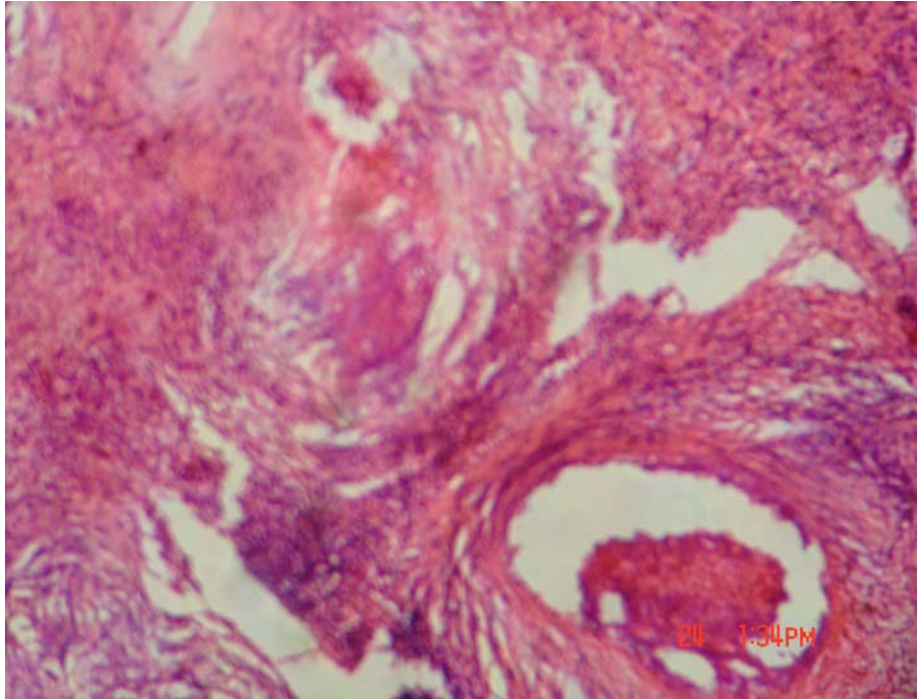
Group of rats inoculated by 3.6×10^7 of *staphylococcus lugdunensis*.

Table (5)

Rat w.	Spleen w	Viable lymph	PFC/3ml	Dead lymph
241	0.546	31,650	4470	7800
233	0.446	28,800	2378	5250
221	0.309	50,250	3315	4500

Table (6) Average of the results of the previous tables.

Species	Rat weight	Spleen weight	Viable lymph	P.F.C/3ml	Dead lymph
<i>Staphylococcus aureus subsp. anaerobius</i>	242	0.554	16,183	6915	
<i>S.aureus</i>	236	0.476	8600	4583	
<i>S.aureus sub spp. anaerobius+S. aurous</i>	245	0.598	20,050	14,738	
<i>S.caseolyticus</i>	244	0.580	23,850	7.536	
<i>S.lygdunensis</i>	232	0.448	36900	3390	



**Fig. No.1 photomicrograph in liver shown multi abscesses :
Liquifactive necrosis surrounded by inflammatory cells ; neutrophils
and mononuclear cells with capsulation. (H&E x10).**

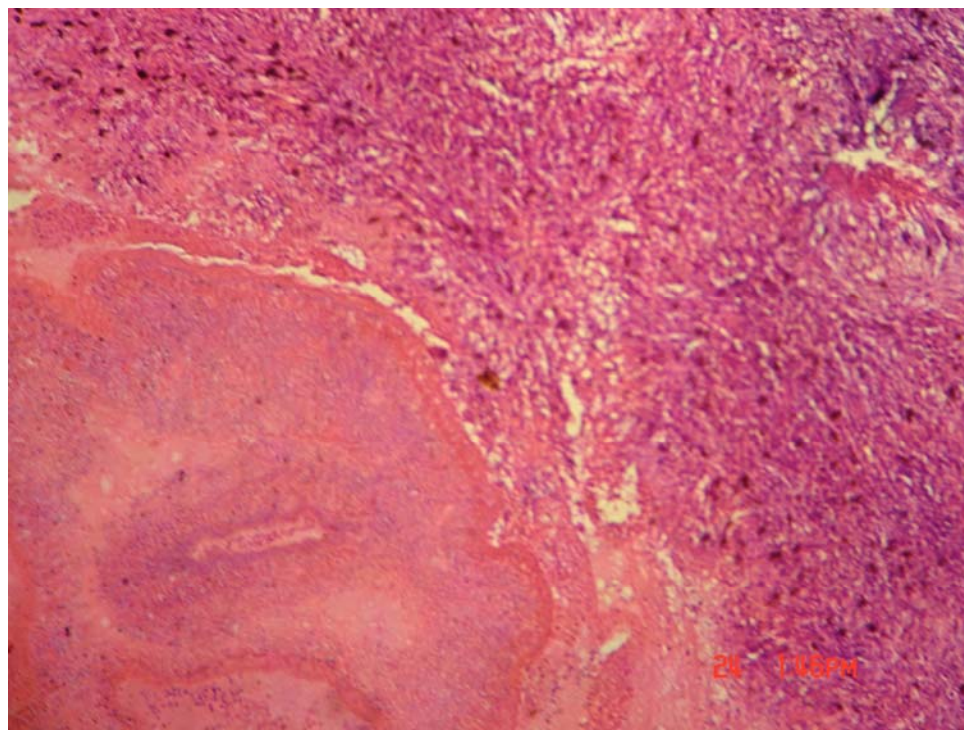
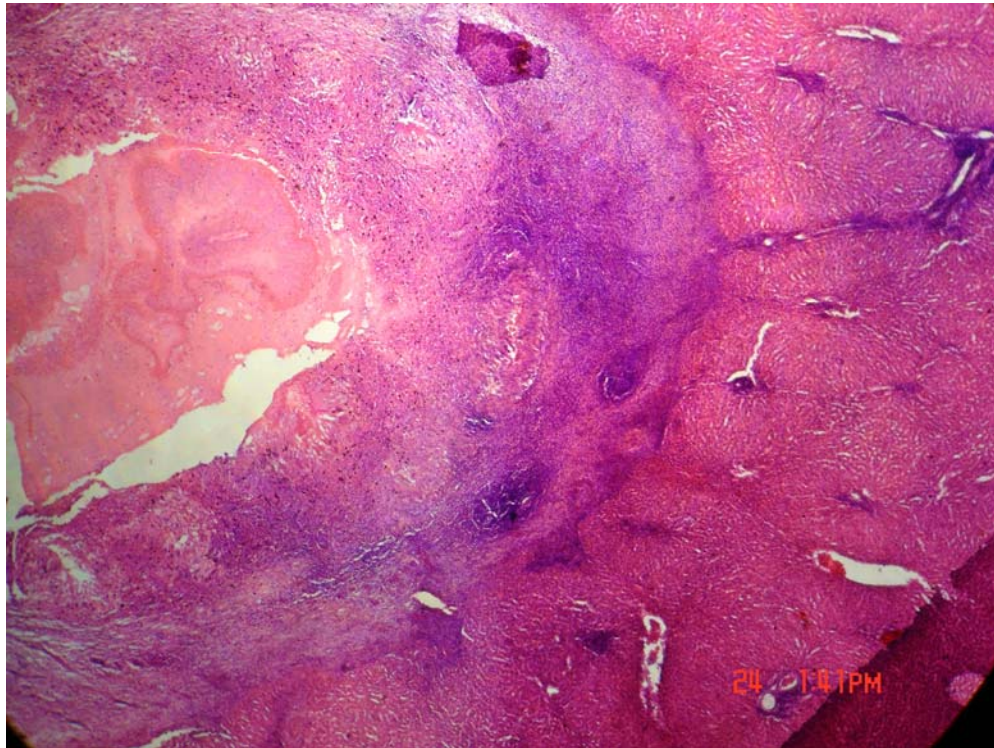
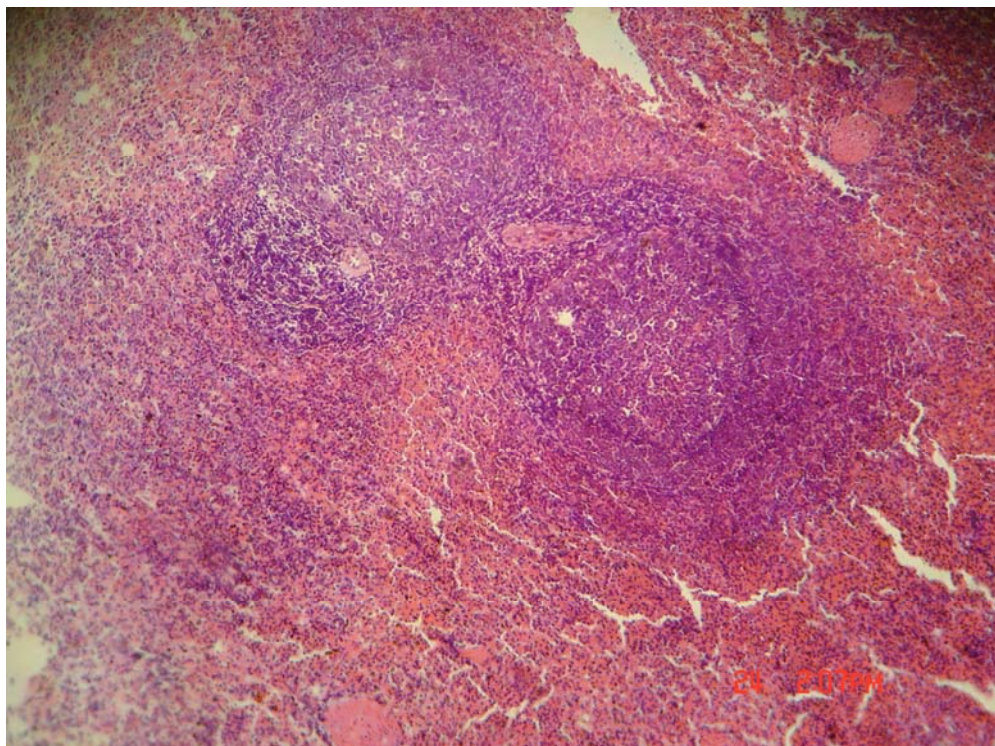
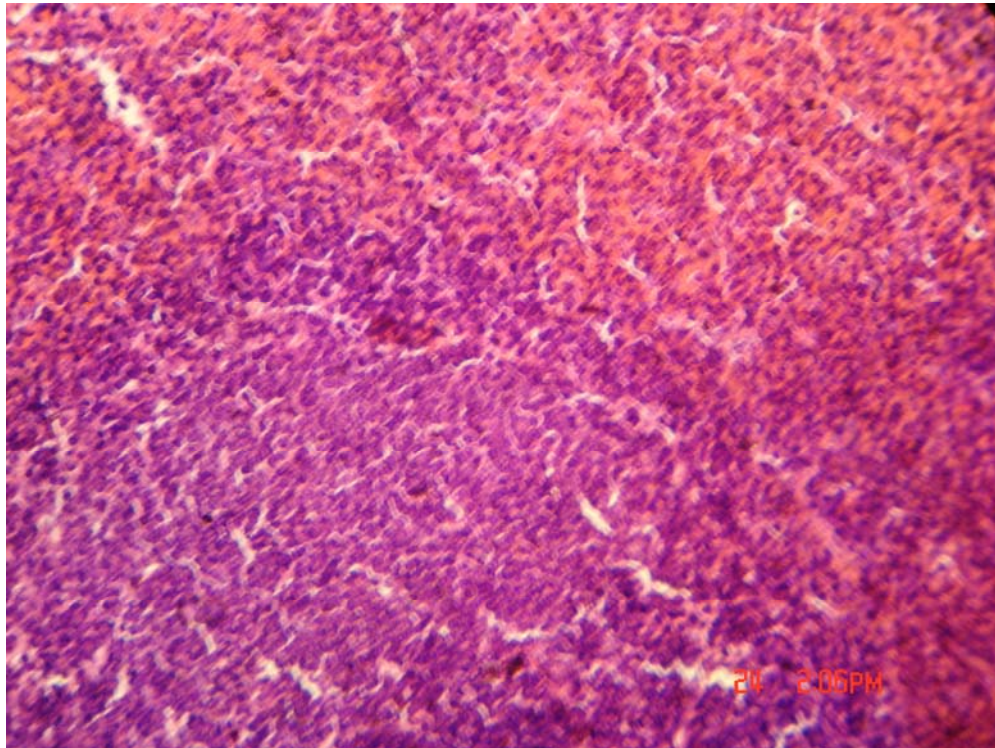


Fig. 2 photomicrograph in liver shown an abscess :

Mature abscess form of liquefactive necrosis with pinkish colour surrounded by inflammatory cells ; neutrophils and mononuclear cells and capsulation. (H&E x10, x40).



**Fig. 3 Photomicrograph in spleen shown suppurative splenitis :
White pulp surrounded by zone filled with infiltration of neutrophils
divided white pulp from red pulp (H&E x40, x10).**

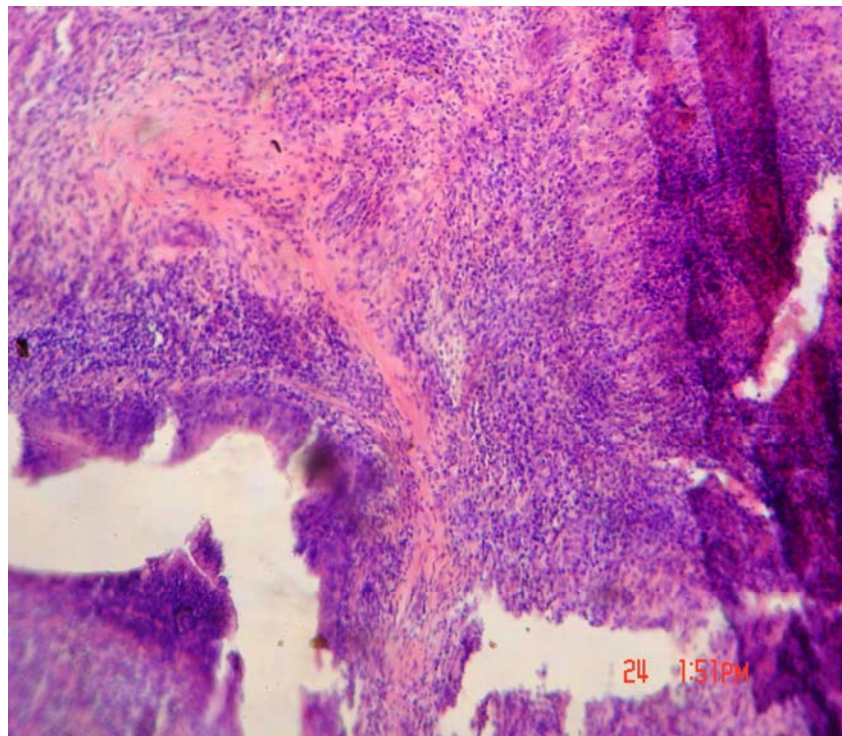
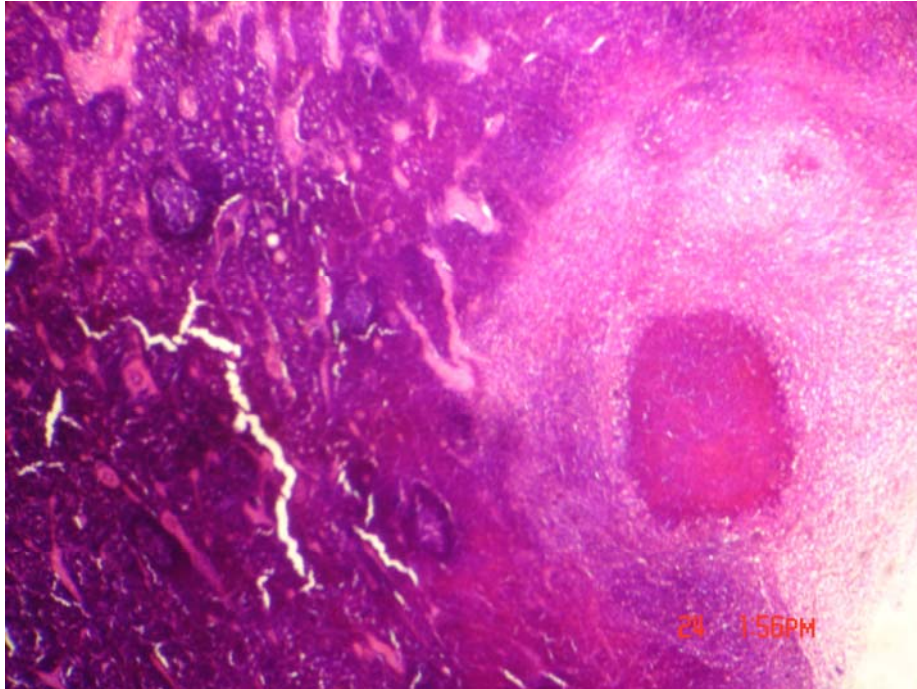


Fig. No.4 Photomicrograph of Lymph nodes :
An abscess form a collection of neutrophils with a very weak
capsulation (H&E x10, x40)



**Fig. No. 5 Photomicrograph in subcutaneous tissue :
Accumulation of inflammatory cells predominantly neutrophils,
mononuclear cells and fiber cells; starting of weak fibrosis.
(H&E x10)**

Discussion :

This study was designed to examine the immunopathological response against Morel's disease in laboratory animals as judged by Plaque forming cell assay (PFCA) as well as the pathology of naturally occurring disease in sheep and experimentally induced on laboratory animals.

In the sheep samples the ruptured abscess appears yellow and homogenous pus, similar results were obtained to those reported with (Morel,1911, Aynaud,1922; Bajmocy *et al.*,1985; Fuente and Suarez,1985 and K. Moller, *et al.*, 2000). Microscopic appearance showed a liquifactive necrosis of non-structured and homogenous, pinkish pus surrounding by inflammatory cells, neutrophils and mononuclear and then are enveloped by capsulation or weak fibrosis.

In this study the infection with whole culture of bacteria revealed positive result of PFCs with untanned RBCs while in other study using vaccine which its protein contents was more than poly saccharide contents, obtained that more plaque formed with tanned RBCs compared to untanned RBCs. The protein antigens combined to tanned RBCs which poly sacchride antigens combined to un tanned RBCS (Hassan,2001).

In this study, the synergetic reaction revealed more plaque formed as compared with the summation result of the cross species between Sasa and *S.aureus* showed in table 6.

In this study notice that the plaque formed when using Killed *S.aureus* with 10 formalin as antigen on sheep RBCs while growing secreted haemolysin which haemolysis sheep RBCs and no plaque formed, however live *Staphylococcus aureus subsp. anaebius* formed plaque when used as antigen indicating that *S.aureus subsp. anaerobius* needed CO_2 to growth and secreting haemolysin.

The PFCA may be the test of choice for detection of anti body producing cells against Morel's disease.

The poly saccharide coat directly to un tanned RBCs.

The isolated organism *staph. aureus subsp. anaerobius* cultured in blood agan and incubated water Co2 tension grew in small grew in small while colonies with partial or complete haemolysis sheep blood agar, the organism appears as Gram positive coccus arranged singly, in pairs or in groups, non motile, uncapsulated and non – sporing, the agreement with Joubert (1958), Fuente, Suarez and Schleifler (1985), Hamad (1989, Hassan (1996) and Karamalla (1997), but Bajmocy *et al.* (1984) observed the organism found more frequently in tetrads and occasionally conglomerates arrangement. El-Sanousi (1989) found that the organism was to be partially acid – fast, Morel (1911), Aynaue (1922 and Carre (1923 a,b) mentioned that the organism didn't grow on simple medial or when incubated aerobically.

In the sheep sample the ruptured abscess appears yellow and homogenous pus, this agreement with (Morel,1911, Aynaue, 1922; Bajmocy *et al.*, 1985; P.Ahrens, N.E. Jensen and Ko , Moller, J.S. Agerholm, P.Ahrens, N.E.Jensen and T.K.Nielsen 2000). Microscopic appearance showed a liquifactive necrosis of no-structured and homogenous, pinkish pus surrounding by inflammatory cells, neutrophils and macrophages with capsulation or weak fibrosis. This agreement with Hamad (1989), K,Morel, J,S,Agerholm, P,Ahrens,N.E.Jensen and T.K. Nielsen (2000), they also mentioned in some lymph nodes trabecular fibrosis, cortical atrophy and chronic inflammation and fibrosis of the capsule occurred due to compression and involvement by an expanding abscess, in some cases, the lymph nodes capsule was located between the lymphoid tissue and the abscess, but in

other it was impossible to determine whether the abscess originated from lymphadenitis or from a perilympho nodular focus.

Other wise in this study. The abscess appear as a collection of inflammatory cells predominantly neutrophils and mononuclear cells, surrounding with a weak fibrosis in this study, the rats were injected intra peritoneally found to be resistant to experimental infection with *S. aureus* subsp. *anaerobius*, *S.aureus*. *S caseolyticus*, *S.lugdunensis*, this agreement with experiment done in guinea pigs and mice by Aynaud (1928), Joubert (1958), Shirlaw and Ashford (1962), Fuente and Suarez (1985) in mice only and agreement with experiment in rabbits by Aynaud (1928) and Joubert (1958). However, Shirlaw and Ashford (1962) and Hamad (1989) observed cellulitis at the inoculation site in two out of four rabbits from which the organism was recovered in pure culture.

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